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# ACTIVITIES REPORT OF THE BASIC AND APPLIED SCIENCES BRANCH DIVISION OF WATER SUPPLY AND POLLUTION CONTROL

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# ACTIVITIES REPORT

## of the Basic and Applied Sciences Branch
## Division of Water Supply and Pollution Control

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Basic and Applied Sciences Branch

Division of Water Supply and Pollution Control

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service

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# FOREWORD

In the U.S. Department of Health, Education, and Welfare the Public Health Service's Division of Water Supply and Pollution Control carries out a program of scientific research broadly directed to: (1) determination of the causes and effects of pollution of the Nation's water resources, and (2) the development of the pollution prevention and control measures necessary to maintain the national water resources in a quality continuously suitable for domestic and municipal water supply, industrial and agricultural purposes, recreation, propagation of fish and aquatic life and wildlife, and other beneficial uses. The research program includes basic and applied research and development and is carried out in cooperation with appropriate public authorities, agencies, and institutions, private agencies and institutions, and individuals. It is necessary that we increase and make use of the research potential of the Nation if we are to solve our water pollution problems within the time limits before us.

In recent years, accelerated population and industrial growth and concentration, new products and technologies, and changing land uses and practices have thrust enormous pollution problems on engineers and scientists who are relatively unprepared because of the limited resources (personnel, facilities, and funds) made available. The research must now deal with new problems caused by the sheer mass of pollutants and by a whole host of new pollutants that are extremely complex in composition and many of which persist in our waters. Pollution may be physical (including thermal), chemical, biclogical, or radiological. Pollutants can include silt from natural sources, synthetic chemicals including detergents and pesticides, biological organisms including bacteria and viruses, organic and inorganic wastes from humans, inorganic wastes such as brines and acids, etc. A pollutant has a deleterious effect on the health, economic, recreational or aesthetic use of water, as well as fish and wildlife.

The analytical tools and scientific knowledge that served well for problems of the past are proving increasingly inadequate for present pollution problems and will become more so with foreseeable future problems. Thus, water pollution research must improve existing technologies and develop new ones while program administrators attempt to hold the pollution line with available knowledge. Methods for measuring costs and benefits related to water pollution and water use must be developed.

Recognizing research needs, the Congress, in the 1961 Amendments to the Water Pollution Control Act clearly indicated its intent that the federal water pollution research program be accelerated. The amendments direct that the Secretary of Health, Education, and Welfare undertake research in three specific areas --the development and demonstration of:

1. Practicable means of treating municipal sewage and other waterborne wastes to remove the maximum possible amounts of physical, chemical, and biological pollutants to restore and maintain the maximum amount of the Nation's water at a quality suitable for repeated reuse;

2. Improved methods and procedures to identify and measure the effects of pollutants, including those created by new technological developments on water uses; and

3. Methods and procedures to evaluate the effects on water quality and water uses of augmented stream flows to control water pollution not susceptible to other means of abatement.

Field laboratories for research, training, and technical services have been planned for six areas of the country: Ada, Oklahoma; Ann Arbor, Michigan; Athens, Georgia; Boston, Massachusetts; Corvallis, Oregon; and Fairbanks, Alaska. Research projects are already underway on pesticide pollution in the southeast, which will continue as a major effort of the Athens facility, and on the effects of pollutants on marine fish, a project at the Oregon State University campus that will be incorporated into the program of the Corvallis laboratory.

Two additional national laboratories have been planned for the research necessary to establish water quality standards for all water uses. These laboratories will be in Duluth, Minnesota, and Kingston, Rhode Island.

To carry out federal responsibility for leadership and accomplishment in water pollution research, and to plan and program research activities, Division of Water Supply and Pollution Control scientists and engineers have defined the following eight broad areas of needed research: (1) identification and measurement of pollutants; (2) effects of pollutants on water quality and water uses; (3) origins of wastes; (4) control of wastes at their source; (5) treatment of wastes; (6) advanced waste treatment; (7) disposal of wastes into the water environment; and (8) water resources conservation and quality management.

Under the auspices of the Basic and Applied Sciences Branch, projects directed to solving problems in these major areas are conducted intramurally at the Robert A. Taft Sanitary Engineering Center in Cincinnati, field laboratories in various regions of the country, Water Quality Research Laboratories, and field locations when and where needed, and by contract with other research and development organizations.

The Research and Training Grants Branch administers a program of grants and fellowships for supporting individual research in the same broad areas of need.

## I. IDENTIFICATION AND MEASUREMENT OF POLLUTANTS

### The Problem

To prevent and control water pollution, it is obvious that we must be able to identify and measure the pollutants that affect water quality and water uses.

In the past, pollution control was principally concerned with the gross effects of the total waste discharge on water quality and seldom dealt with the specific components in the waste. Laboratory determinations, therefore, were devised and used, such as dissolved oxygen, biochemical oxygen demand, suspended solids, total solids, and nitrogens (free, albuminoid, and total) that identified certain effects and gross amounts of pollutants; rough determinations of toxicity to fish of certain suspected chemicals and wastes were made. Few of these laboratory methods were specific for individual pollutants, nor did they then need to be.

All this has changed in recent years. Municipal sewage has changed in character, composition, and strength. Today's conventional treatment processes are increasingly ineffective for the stronger, more complex, and larger volumes of municipal sewage; it is necessary to detect and measure the individual components in sewage if new and effective treatment and controls are to be developed.

Industrial wastes too have changed dramatically. Not only has accelerated production resulted in an increase in the common industrial wastes but process changes have altered their characteristics. Even more difficult, changing technologies are producing a wide variety of wholly new synthetic products and wastes of which little is known. A tremendous increase in the use of agricultural chemicals is creating a new land drainage pollution problem. Nuclear power developments and the growing use of radioactive materials in industry, medicine, and research may produce still other wastes that are undetected by the usual methods employed to assess water and wastes.

Present methodology of detecting and measuring the presence and amounts of significant pollutants in water is particularly deficient. A whole new arsenal of analytical tools must be developed soon if we are to be able to adequately assess the water pollution problem, predetermine potential chronic toxic effects of pollutants, and devise effective, efficient programs and treatment methods for preventing and controlling pollutants.

### Intramural Projects

(1) Improvement of procedures for recovery, identification, measurement, and evaluation of organic contaminants affecting water quality.

(a) Sampling methods: The major portion of this program of investigation of organic contaminants has been based on the use of an activated carbon filter to isolate sufficient samples from surface waters to serve laboratory requirements. The efficacy of this procedure in collecting odorous

contaminants was proved high. Further studies were, however, required to evaluate the procedure as a quantitative basis for determining the total organic chemical content, as in water quality monitoring. An intensive study was made of the effect of varying water to carbon ratio and retention time of water in the column; this study resulted in a modification of the operating conditions whereby the recovery of total organic matter is doubled compared with the previous operating conditions. More valid quantitative data are obtained.

The development of very sensitive analytical instruments has made it possible in certain cases to eliminate sampling by carbon adsorption. Instead, a water sample ranging from a liter to several liters is shaken with a low boiling, immiscible solvent. The solvent, e.g. ether, is evaporated to less than a milliliter and then analyzed by gas chromatography. This method was applicable for the determining of certain pesticides and for monitoring the occurrence of petrochemical wastes in a highly polluted river.

In the opposite direction, where very large samples are required for physiological testing or for very extended laboratory investigations, a large-scale filter assembly mounted on a trailer has been developed. Application of this equipment has now reached the limit of capacity of the corollary extraction equipment. A contract was issued for the design and contraction of a second extractor assembly of improved efficiency. This transportable equipment, to be delivered early in Fiscal Year 1964, is designed for use in field laboratories.

(b) Odor research: The phenomena of odor addition and synergism, previously demonstrated in an arbitrary laboratory situation, have been further studied in terms of a real stream pollution condition. A majority of the odorous organic contaminants were identified and measured. Addition and synergism of the individual odors of all the components increased the threshold odor of the group.

Investigations were begun to test for the same phenomena in odorants at concentrations above the odor threshold. The first stage of this study has been a search for a valid measure of supra-threshold odor intensity, superior to existing subjective scaling methods. The search was in the direction of physical analogs. Results have not attained the reliability necessary for the more complex, succeeding stages of the study.

(c) Pesticide analysis: A very sensitive method was developed for the analysis of many common chlorinated insecticides in water by gas chromatography. The method applies to concentrations below those that kill fish. The method was tested for its value in studying fish kills in surface waters rich in other dissolved organics. Under these conditions, both positive and negative

interferences sharply limit the utility of gas chromatography. A comparison of this method was made with two others: paper chromatography and infrared spectrometry. The last is most specific, but far too insensitive. All methods have defects and do not meet requirements for water quality control purposes. Improved specificity is the present objective.

(d) Carcinogens: Repeated trials with unactivated wood charcoal and graphite (the least active carbons obtainable) showed an "irreversible" adsorption of benzpyrene on the order of 0.2 µg benzyprene per gram of adsorbent. Certain plastics, cellulose triacetate in particular, proved to have excellent adsorptive properties, however, and preliminary trials indicated that quantitative or nearly quantitative desorption with methanol was possible at loadings on the order of 5 µg per gram of plastic.

Further investigation is planned at very low loadings with a fluorescence spectrophotometer, which is expected to give much greater sensitivity and be less subject to interference than the ultraviolet method now in use.

(2) Development of analytical methods in connection with Advanced Waste Treatment Research Program.

(a) Chemical oxygen demand: A modified COD test has been developed that employs a mercury salt to tie up the chloride ions and thereby eliminates the customary chloride interference. The procedure is published in the July 1963 issue of Analytical Chemistry.

(b) Foamability: A physical response method for estimating and comparing foamability has been developed. This property of waste water has proven troublesome in several treatment processes and was not satisfactorily correlated with ABS content of the water.

Service Program

Facilities and personnel continued to be made available to various state and local public health agencies. These activities resulted in an early acquaintance with new problem areas demanding research attention. Some of the service activities were:

(1) Petroleum contaminants were characterized in a carbon filter extract of Lake Michigan water at Chicago. The oil, which contained kerosene, was of sufficient concentration to cause the intermittent taste and odor problems observed.

(2) A fish kill in the Roanoke River in North Carolina was investigated by means of carbon filter sampling and by direct analysis of water samples. Insecticides, originally suspected as a cause, could not be clearly demonstrated. Dieldrin was the only probable one, and its

concentration was too low to kill fish. Resin acids were, however, present at about eight times the level lethal to fish, and extract from the filter was correspondingly toxic. Information in the state's possession, supplemented by the above, established the source of pollution as a kraft-process pulp mill.

(3) A die-off of ducks at Lake Holly, Virginia, was the basis of cooperative work with the Fish and Wildlife Service. Insecticides were suspected, and analyses were made by methods of gas chromatography then under development, and by paper chromatography. Insecticides could not be demonstrated, and the project showed the weakness of current methods when applied to moderately or heavily contaminated waters. Another possible cause was thought to be petroleum materials discharged by a sewer; mud from the lake nearby was rich in waste lubricating oil. Pathological studies by the Fish and Wildlife Service showed, however, that a more probable cause of death was infestation of the ducks with a nematode.

Contract Projects

(1) Development of a new technique for determining organic contaminants in water.

The Dow Chemical Company began evaluating their total carbon analyzer as a useful, instrumental method of analysis of the organic material entering and leaving waste water treatment processes. A procedure was developed to handle the organic solids in various streams so that they would also be analyzed. Since the instrument measures total carbon, several methods were devised to remove carbon dioxide and bicarbonates without removing volatile, carbonaceous constituents of various, typical waste-water streams.

(2) Assay of radioisotopes in sewage.

The California Institute of Technology has progressed rapidly in developing an analytical technique for measuring the amount of low energy radionuclides in water and sewage. The successful development of this procedure will aid in monitoring waters for the presence of very low concentrations of these radioactive pollutants that may eventually be of significant importance to health.

## II. POLLUTION EFFECTS ON WATER QUALITY AND WATER USES

### The Problem

Standards of water quality are related to the water use. Only when we know with a greater degree of confidence the quality requirements for the various water uses can we fully safeguard water users, design the kinds of treatment facilities that will meet these standards effectively and economically, and understand the changes that are deteriorating water resources.

Many new wastes, particularly in the synthetic chemical field, are being discharged into waters where they appear as discrete pollutants. Little is known of their effect on water quality except that they are extremely persistent and stable in water and resist removal by conventional water and waste treatment methods. Some cause tastes and odors in drinking water, others are toxic to fish and aquatic life or taint fish flesh, and very little is known of their toxic effect on humans except that present concentrations in natural waters do not appear to be acutely toxic. Methods are needed to measure the pollutional characteristics of new wastes where traditional parameters do not apply - methods that will accurately measure the effects, including chronic effects, of new pollutants on water quality and suitability for use. Methods are needed also for some of the older types of wastes and for natural pollutants whose pollutional effects have not been fully assessed.

### Intramural Projects

(1) Algae and other interference organisms in water supplies.

Studies of cultures of blue-green algae susceptible to the algal virus indicate that they all belong to one species rather than to several taxonomic groups. This species is universal in distribution and one of the common forms encountered in water.

Information from 135 workers has now been filed on pollution-tolerant algae; this involves approximately 800 species and varieties.

The rare blue-green algae Lyngbya circumcreta, reported previously from Africa and India, was identified as the probable cause of a severe odor problem in a water system in Louisiana.

(2) Algal growths in surface waters.

A tentative key to the species of Sceanedesmus was made, (for use) pending completion of descriptive records of all species. This is a large genus with approximately 175 species. An immediate result of the study has been the discovery of two species, common in sewage ponds, that have not been previously reported from this continent.

(3) Determination of the environmental requirements of plankton organisms through physiological, toxicological, histological, biochemical and ecological studies.

(a) Nutritional requirements of plankton organisms.

The addition of a common household detergent to Chlorella pyrenoidosa resulted in an increased rate of cell division as well as a decreased size of the cells. Of the several ingredients in common household detergents, only sodium tripolyphosphate was found to be responsible for these changes. When orthophosphate was substituted for sodium tripolyphosphate, it was found that the orthophosphate did not have the same properties of sodium tripolyphosphate for stimulating cell division.

An attempt was made to determine the minimum concentration of both phosphorus and nitrogen needed to propagate Chlorella pyrenoidosa. Cells cultured in a phosphorus and nitrogen-free medium for 8 days showed, however, that cell storage of these nutrients was enough to permit a tenfold increase in growth in 140 hours with no nitrogen and 2.4 milligrams of phosphorus per liter.

(b) Physiological activities of plankton organisms.

Culture studies of Chlorella pyrenoidosa clearly showed that this alga was able to store phosphorus and that this stored phosphorus was available for growth when phosphorus was not supplied from the environment. When cells depleted of stored phosphate were added to phosphate-free medium, there was no growth of the organisms. This would clearly indicate that phosphorus is necessary for the nutrition of this alga.

By the use of a photosynthetic Warburg apparatus, it was shown that, at a saturated light intensity of 1600 ft-c, the optimum temperature for photosynthesis in the diatom Navicula minima is 31° C, while that of the green alga Chlorella pyrenoidosa is 35° to 37° C.

(c) Effects of pollutants upon plankton organisms.

Growth of Chlorella pyrenoidosa was determined in the presence of 0, 5, 10, and 20 milligrams of zinc per liter (mg/l) (as zinc sulfate). There was no algal growth in 10 and 20 mg/l zinc and only limited growth in 5 mg/l zinc. Further study revealed that growth was inhibited with zinc concentrations as low as 2.0 mg/l.

(4) Determination of the environmental requirements of benthic organisms through physiological, toxicological, histological, biochemical, and ecological studies.

(a) Determination of minimum dissolved-oxygen (DO) concentrations tolerated by aquatic invertebrates and the effects of temperature and other factors on oxygen requirements.

A special apparatus was constructed that provided continuous flow and constant, reduced oxygen concentrations in the test chamber.

The oxygen concentrations at which mortalities occurred were recorded for the nymphs or larvae of nine species of mayflies, three species of stoneflies, one species of caddisfly, three species of aquatic flies, four species of Odonata (dragonflies and damselflies), four crustaceans, and four miscellaneous aquatic invertebrates. It was soon learned that a few hardy individuals of a generally recognized sensitive mayfly species, for example, might exhibit tolerances as small as 1.0 mg/l DO, or slightly less. Several runs with the same species have been made, therefore, to determine the oxygen concentration at which most specimens of a given species died. For example, with the mayfly Isonyohia bicolor some individuals survived concentrations less than 1.0 mg/l DO, but most of them died at concentrations close to 3.0 mg/l.

(b) Determination of the acute and long-term toxicity of selected pesticides to aquatic invertebrates.

A series of continuous flow-through toxicity tests of DDT were carried out in 10 oval tanks, each 3 feet in length, in which water currents simulating those found in streams were produced with paddle wheels. A variety of bottom organisms were used in each run.

Test concentrations of DDT ranged from 0.5 mg/l to 0.03 mg/l. Mayfly nymphs of the species Stenonema ares, S. heterotarsale, Isonychie bicolor, and Ephemerella sp. were immobilized or killed within a 3-day period by 0.03 mg/l of DDT, the smallest concentration used. Fingernail clams Sphaerium striatinum, snails Goniobasis sp. and water pennies Psephenus herricki, survived concentrations of 0.25 mg/l; about half of the caddisfly larvae of Cheumatopsyche were killed by concentrations of 0.03 mg/l. The above DDT values were calculated rather than analytically measured. The DDT was dissolved in acetone and emulsified with Sterox before being dispersed in the test water.

(c) Effects of detergent on stream bottom animals.

Water containing 32 mg/l of alkyl benzene sulfonate (ABS) obtained from a packaged detergent (27% ABS) totally eliminated the mayflies Stenonema ares and S. heterotarsale, the fingernail clam Sphaerium striatinum, and the snail Goniobasis sp. At concentrations of 16 mg/l, S. ares, S. heterotarsale, and Sphaerium were seriously reduced. Concentrations of 8 and 4 mg/l resulted in only slight to no noticeable effects.

When a product composed of 92.5 percent ABS was used, the ABS produced more drastic results; 8 mg/l eliminated both species of mayflies and resulted in large losses of Goniobasis. Even 4 mg/l caused high mortality to the mayflies. In all cases, caddisfly larvae of Cheumatopsyche seemed relatively unaffected.

(d) Determination of the physiological responses of crustacea to sublethal concentrations of heavy metals, especially effects on growth and development.

Long-term studies with the stream crayfish Orconectes rusticus indicate that the effects of copper will vary according to stage of the life cycle. Animals were exposed in a continuous-flow apparatus that allowed molting and growth data to be gathered on individual animals. Rapidly molting juvenile crayfish and newly hatched young were found to be approximately 20 and 50 times, respectively, more sensitive than adults under similar conditions. Fifty percent of the newly hatched young were killed in less than 24 hours in a concentration of .125 mg/l copper. Embryological development is affected in concentrations as low as .06 mg/l. Growth during the first month after hatching is retarded in a minimum concentration of .015 mg/l. Other experiments have shown that irreversible effects are produced by exposure to copper for very short periods of time. The effects of copper may be cumulative as shown by interrupted exposures to very small concentrations over several weeks.

(5) Determination of environmental requirements and safe concentrations of wastes and other materials for fish under conditions of continuous exposure.

(a) Determination of the toxicity to fish of various pollutants.

Amitrol-T, which is used as a bud inhibitor, is very effective when used with 2,4-D to control water hyacinths. Single applications of only a few pounds per acre of these chemicals, in combination, have done the work of several applications of 2,4-D in earlier treatments. Fenac has been found effective for the control of Eurasian milfoil Myriophyllum spicatum, and the Hyamine compounds have been found useful in the destruction of algae that are difficult to control. Amitrol-T was weakly toxic to both bluegill sunfish Lepomis macrochirus and fathead minnows Pimephales promelas with 96-hour $TL_m$ values of 440 and 118 mg/l, respectively, in soft water.

Fenac was more toxic to fish with the following 96-hour $TL_m$ values in soft water: bluegills - 13.0 mg/l; fatheads - 11.1 mg/l; and in hard water: bluegills - 66.0 mg/l; fatheads - 32.0 mg/l.

Hyamine 3500 was highly toxic to bluegills. The 96-hour $TL_m$ values were 0.69 mg/l in soft water and 1.10 mg/l in hard water.

Studies on the toxicity of ABS to the eggs and fry of fish were begun at the start of the spring spawning season in 1963, and the results, thus far, indicate that the fry are much more sensitive than either the eggs or adults.

A series of six 3-week tests was completed to determine the effect of DO on the toxicity of zinc in the sublethal ranges. Studies of mortality, growth, and blood characteristics indicated that a reduction of DO from 6 to 2 mg/l increases the toxicity by a factor of 2.

A concurrent series of four tests on the chronic toxicity of zinc and copper, singly and in mixtures, was initiated in December 1962. Very young fathead minnows are being used, and the effects on reproduction and growth are being studied. The fish have reached sexual maturity and will be spawning soon. Rather pronounced synergism has been found in long-term effects, contrary to recent findings by other workers.

Studies of the toxicity of zinc to an ecosystem in artificial streams are in progress. The movement, distribution, and accumulation of zinc are being studied along with the biological effects; thus far the most striking result has been the extreme sensitivity of the plankton to zinc.

Growth rates of cichlids, kept on an unrestricted diet of tubificid worms, in continually renewed sodium cyanide solutions increased temporarily at very small, sublethal cyanide concentrations and were only moderately reduced at larger concentrations that were not much below the lethal level. Even at the large concentrations, food consumption rates increased but food conversion efficiencies were depressed. Growth rate changes that occur with increased exposure time indicated acclimation. The swimming performance of the fish was more or less impaired at sublethal cyanide concentrations; the degree of impairment varied not only with cyanide concentration and exposure time but also with the kind of performance measured. Thus, the maximum sustained swimming speed and the duration of swimming at much greater speed were not as markedly reduced after prolonged exposure to small cyanide concentrations as was the duration of swimming at an intermediate speed. In an experiment in which cichlids were starved at sublethal concentrations of cyanide, the energy reserves of the experimental fish (calorific values) declined more rapidly than those of starved controls, at least initially. Cyanide concentrations near 0.02 mg/l had no apparent effect on the behavior and reproduction of cichlids, and even a concentration of 0.1 mg/l, which is only slightly below the lethal concentration for juveniles, evidently affected only the survival

and feeding of young fry, which hatched successfully from eggs spawned in aquaria at this concentration.

Studies of the nature of the toxic constituents of wastes from northwestern, kraft-process pulp mills pulping Douglas fir have been directed mostly toward better evaluation of the relative importance of the toxic constituents that are readily removable by aeration. The contribution of this "volatile" fraction of whole mill effluents (from 3 mills) to the toxicity to guppies has been found to vary greatly from mill to mill and from time to time, but the removal of these substances usually does not reduce the toxicity of the effluent samples greatly.

(b) The development and testing of autopsy techniques for determining the cause of death in pollution killed fish.

An autopsy method for zinc-caused fish mortality has been successfully developed. Five species of fish were killed in experimental exposures, and the subsequent autopsy indicated the validity of the method for those species. Over 1000 individual fish, consisting of 22 species, have been collected from a variety of streams and lakes. These specimens were analyzed for zinc to obtain data for normal fish and to establish the variability to be expected. This method is specific for zinc, can be performed on decomposing fish, and is not affected by the presence of small amounts of other toxicants or disease.

Currently, work is progressing on an autopsy for cadmium and copper.

(c) Determination of the influence of dissolved oxygen upon the survival, development, growth, and movement of fish.

In repeated experiments with juvenile coho salmon, the appetite and growth rates of salmon held at 18° C on unrestricted rations of tubificid worms at nearly constant oxygen concentrations declined when the oxygen concentration was reduced below the air-saturation level. The percent gains in weight of the fish in 18 days declined by one-half at oxygen concentrations near or slightly below 3 mg/l. Concentrations more than three times the air-saturation level had only a slight depressing effect on growth. Growth was markedly impaired by wide diurnal fluctuations of oxygen concentration, even when the arithmetic and geometric mean concentrations fell within the range of favorable constant concentrations (i.e., those at which growth was nearly as good as it was at the optimal constant level). Coho salmon kept on equal, restricted rations at different, nearly constant oxygen concentrations showed an appreciable impairment of growth.

Free carbon dioxide concentrations up to 40 mg/l had no pronounced depressing effect on the maximum sustained swimming

speed of juvenile largemouth bass at any dissolved oxygen concentration tested at 25° C, although the speed was markedly reduced at oxygen concentrations much below 5. Increased carbon dioxide concentrations near 20 had little effect on the sustained swimming speeds of juvenile coho salmon at various dissolved oxygen concentrations, at 20° C, especially after brief, 20-hour, acclimation of the fish to the elevated carbon dioxide levels. Yet any considerable reduction of oxygen concentration below the air-saturation level resulted in reduction of the swimming speed. Carbon dioxide concentrations near 50 had a considerable effect even after 20-hour acclimation; this effect was most pronounced at high oxygen concentrations and slight or absent at very low oxygen concentrations near 2.

Experiments on the influence of continuous exposure to reduced oxygen concentrations during and after embryonic development and on the postembryonic growth of coho salmon and steelhead trout demonstrated impairment of the growth of sac fry reared at reduced dissolved oxygen concentrations. Not only was complete absorption of yolk delayed, but also the size of the fry at the time of complete yolk absorption, i.e., the maximum dry weight of unfed fry, was reduced, which indicated reduced efficiency of utilization of yolk for growth. The effect of moderately reduced oxygen concentrations on the coho salmon fry was greater than the effect on steelhead trout fry; the latter showed pronounced reduction of the maximum attained dry weight, not including weight of yolk, only at the lowest tested oxygen concentration, about 2.9.

(6) Role of water in transmission of viral diseases.

(a) Detection of minute amounts of virus in dilute suspensions.

Studies to concentrate viruses from large quantities of water by the suspended cell technic have been hampered by the difficulty of retaining cells on filters without clogging them and concurrently passing large volumes of water through the cell suspension. Efforts have been made with several types of filters of varying porosity and with alginate cotton; the cotton is used both as a pad for cells and also as a primary adsorbent. Studies are underway to determine the stability of several enteroviruses as a preliminary step in the study of adsorption and to evaluate these viruses in the alginate system.

Additional success was achieved recently with very fine glass beads that were used both as a support for the cells and as spacers among the cells. Work with this last system indicated that the cell and bead concentrations may prove to be critical if clogging is to be avoided, and, therefore, further efforts with this system will take this attitude.

Efforts to concentrate viruses from large quantities of water by filtration through membrane filters have met with some success, too. It has been demonstrated that 99 percent of the virus filtered through a 50 millimeter membrane is retained in the filter.

Since alteration of pH might result in the adsorption and elution of viruses in the alginate system or in elution from the membrane filters, it was necessary to establish virus resistance at various pH levels, including those achieved when water is treated by softening procedures. The pH studies have been undertaken with a poliovirus type I and an echovirus type 7 strain. The data indicate that the poliovirus stability is greatest at pH 7 and falls off with increasing speed at both sides of neutrality. A predictable pattern of destruction occurs between pH levels of 7 and 9.6, but above 9.6 the die-off rate becomes disproportionately rapid. At pH 9.6, 11 percent of the virus is destroyed in about 3 days. Studies with echovirus 7 indicate that it is more sensitive than the poliovirus to high pH levels. There is, thus, some indication that water softening procedures may be of some value in reducing the virus levels of water supplies.

(b) Value of coliforms as indicators of viruses in water.

Data have been obtained on the survival times of three indicator bacteria, Aerobacter aerogenes, Escherichia coli, and Streptococcus faecalis, and four enteric viruses, poliovirus type I, echovirus types 7 and 12, and coxsackie virus A9, in various waters. Survival times of these seven organisms have been obtained in raw septic tank effluent and effluents sterilized by autoclaving or filtration. The results indicate that, in general, survival times of all organisms are shortest in the raw septic tank effluent, with the viruses surviving about twice as long as the bacteria. Lowering the storage temperature from 27° C to 4° C resulted in an approximate fivefold increase in survival times of both bacteria and viruses. Autoclaving the effluent increased bacterial survival times considerably at 27° C, but had no effect on virus survival times. Sterilization of the effluent by filtration had essentially no effect on survival times of any of the organisms. The mechanisms of inactivation are apparently complex and are dependent on the type of water, temperature, type of organism, and other factors yet to be delineated.

(c) The value of the Hyperion process in removing viruses from water.

A total of 174 samples, collected at several different points from the experimental Hyperion ground-water-recharging project in Los Angeles, California, have been examined for viruses. Of about 70 samples collected from June to October 1962, only five raw-sewage samples were found to contain viruses. At about this time it was announced that the County of Los Angeles was to embark on a mass poliomyelitis immunization program with the use of live virus and it was decided to continue the virus testing

program until several weeks after the last dose of vaccine was administered. This plan allowed testing of the entire Hyperion treatment processes under extreme virus-loading conditions not likely to ever be encountered under "normal" conditions. After the immunization program, virus was found not only in raw sewage but in the activated sludge plant effluent and in such effluent after it had been passed through a rapid sand filter. No virus was ever detected, however, in the recharge water, diatomaceous earth filter effluent, or in the sampling wells located from 20 to 300 feet from the recharge point. It appeared that the process effectively removed at least the three types of poliovirus from sewage.

(d) Prevalence of poliovirus in sewage and the Ohio River after oral vaccine administration.

Oral poliomyelitis immunization of approximately 70 percent of a population whose sewage is treated by one local primary treatment plant offered a unique opportunity to follow poliovirus through this sewage treatment process and in the Ohio River, which received the treated sewage. Weekly samples were collected from both influent and effluent of the plant by both grab and gauze-pad methods. Additional gauze-pad samples were collected from the Ohio River at points 50 to 1000 feet below the treated sewage outfall. Results indicated that virus was present in considerable quantity in sewage 5 days after vaccine administration. Virus, although not necessarily poliovirus, continued to be detected in some quantity in sewage 9 months after the last dose of vaccine was administered. Virus was detected in the river as far as 1000 feet downstream from the sewage outfall.

(7) Significance of protozoan and metazoan organisms in water.

Free-living amoebae, ciliates, flagellates and rotifers are the predominant groups of zoological microbes in natural waters besides the free-living nematodes. Because many of them are bacteria feeders, they are found in very small numbers in clean waters. Their numbers increase when the water receives surface drainage and markedly increase when it receives effluent from aerobic sewage treatment. Experimentation has produced no evidence that free virus particles are taken in by rotifers and ciliates, apparently due to the inability of their cilia to concentrate such small particles. On the other hand, tissue debris from cell cultures infected with echovirus type 7 are ingested by free-living amoebae; but the virus embedded in the debris is quickly digested after the ingestion. These and other results indicate that reduction of human enteric pathogens by zoomicrobes is unlikely to be of significance at concentrations normally present in sewage and that free-living nematodes of sewage-treatment origin are unlikely to be carriers of such pathogens under normal conditions.

If control of nematodes in water supply is desired, the most effective way of doing it is to control them while they are still in the sewage effluent prior to its discharge into the receiving water. A good chlorination process can facilitate 90 to 95 percent removal by immobilizing and subsequent settling of the nematodes to the bottom of a detention tank. Some organic phosphates are effective in destroying all stages of nematodes at a concentration as low as 20 mg/l; but the undesirability of the presence of organic phosphates in water, even in trace amounts, makes their use in this respect unsuitable. They can be used effectively, however, in irrigation water to control nematodes that are parasitic to crops. Stabilization ponds are effective in controlling nematodes that are bred in primary or even secondary treatment processes.

These groups of zoomicrobes can also be used in examination of water for its sanitary quality. Protozoa have been considered for years as possible pollution indicators but a practical procedure has not been found to distinguish pollution tolerant species from intolerant species. The zoomicrobes, however, can be used for that purpose on a quantitative, rather than qualitative, basis. Preliminary study showed that the number of zoomicrobes compared with the number of pigmented protozoa can be a reliable indication of pollution by effluent from aerobic treatment.

Free-living nematodes have also been studied for their possible use as an assay organism for staphylococcal enterotoxin. Results indicated that they are so resistant to the toxin they can live on it as a source of food.

(8) Cultivation, enumeration, and control of nuisance slime organisms.

A medium, modified from one recommended by Kadota and coworkers in Japan, was found that was suitable for the quantitative determination of the most probable number (MPN) of sulfate-reducing bacteria by use of the multiple-tube technique. The quantitative estimation of the number of organisms present in samples can be extended to levels below one organism per 100 milliliters of sample by filtering large volumes of the sample through a membrane filter and aseptically placing this filter in a screw-cap tube containing the medium for the growth of the sulfate-reducing bacteria. To test the suitability of this enumeration procedure for natural samples, a series of samples from four rivers, one creek, seven wells, one spring, influent and effluent from a sewage treatment plant, and tap waters was examined. The numbers of sulfate-reducing bacteria present varied from 0.045 per 100 milliliters of tap water to $7.9 \times 10^6$ per 100 milliliters of sewage effluent.

A microscopic examination of a manganese deposit removed from the distribution system of a waterworks system obtaining its water from deep wells in the alluvial plain near the Greater Miami River showed forms morphologically resembling iron bacteria. Deposits of

Gallionella had also been removed from a pipe joint in the distribution system. Samples of water from the wells and from the distribution system were examined for the presence of iron bacteria, numbers of fecal coliforms, most probable numbers of sulfate-reducing bacteria, total count of bacteria on TGE agar, pH, dissolved oxygen content, oxidation-reduction potential, chemical oxygen demand, and the content of manganese and iron. Floccules consisting of filamentous bacteria and manganese and iron were found in the water from the wells. The organisms present resembled Gallionella, Leptothrix, and either Crenothrix or Clonothrix. Fecal coliforms were less than one per 100 milliliter of sample and the MPN of sulfate-reducing bacteria was 33 per 100 milliliter of sample. The total count of nonfilamentous forms on TGE agar was 10 bacteria per milliliter of water sample from the wells. The pH of the water was 7.3, and the dissolved oxygen was close to zero in the water from the wells. The manganese content was 0.23 mg/l and the iron content 0.05 mg/l. A study of photomicrographs of the different types of iron bacteria found in the deep wells revealed a filamentous form resembling Leptothrix but, when isolated in pure culture, proved to be a spore-forming Bacillus species. Attempted cultivation in a medium prepared with filtered water from the wells failed to grow the original forms found in the water samples.

The methods described in "Standard Methods for the Analysis of Water and Wastewaters" for the cultivation of iron bacteria have been unsuccessful. Phase contrast microscopy is the best tool for the identification of these organisms but is subject to error due to the difficulty of interpreting morphological observations. When the true iron bacteria are grown in pure culture, the factors influencing their development may be systematically studied to initiate a control program.

(9) Epidemiological significance of water pollutants.

A compilation was made of waterborne disease outbreaks in the U. S. for the period 1946 to 1960. These outbreaks have been analyzed for the type of water used and the cause of the failure to provide safe water. Comparisons were made of the total reported enteric disease incidence with that of diseases associated with waterborne outbreaks. Data about waterborne diseases will be currently maintained, and trends of causes will be analyzed.

Other socio-economic and environmental factors were investigated that could influence the correlation of dissolved solids in drinking water with cardiovascular disease mortality. It was noted that the following factors were also correlated with cardiovascular death rates: population size of metropolitan area; density, i.e., population per square mile, percentage of population same house (1949 to 1950), percentage foreign-born in population, percentage non-white in population; elevation above sea level, physicians per 100,000 population; and median family income.

Other factors investigated to date that did not correlate with cardiovascular death rates were: persons per household, school years completed, average annual temperature, and annual precipitation.

The interaction of these factors with water quality parameters is being investigated.

Contract Projects

(1) Identification of relationships between exposure of fish to waste chemicals and the degree of subtle physiological response of fish to these toxicants.

Engineering-Science, Inc. is investigating parameters of biological activity to aid in the selection of those that would be meaningful in demonstrating the toxic effect of pollutants at very low concentrations. The parameters of conditioned response, conditioned avoidance response, rate of regeneration of tissue, and total activity of the organism were selected for further study, and an apparatus was designed and constructed to allow quantitative measurement of the response. In preliminary testing of the methods and apparatus, organic insecticides were chosen as the toxicants. The results showed: (1) Conditioned response is a quantifiable parameter and shows promise in the development of practical tests for toxicity. (2) Toxication due to organic insecticides alters the degree of conditioned-response acquisition patterns in proportion to the time-concentration function of exposure. (3) Conditioned avoidance response can be quantified and, although more complex, may be more useful in ecological evaluation of the parameter. (4) Regeneration in flatworms is adaptable to bioassay studies; however, histological studies of toxicated organisms are needed to determine more accurately toxic effects, since visual observation of regeneration time was found to lack sensitivity. (5) Total activity appears to offer promise as a quantitative measure of toxic effects of compounds whose toxic natures are undefined. (6) Facilities and methodology have been developed to produce a standard genetic strain of experimental fish, with opportunity for controlled production of strains possessing desired experimental characteristics. In addition to the economic benefits that accrue from this operation, the reduction in genetic heterogenicity prevalent in commercial stocks is of scientific benefit.

## III. ORIGIN OF WASTES

### The Problem

Our ability to determine the source of pollutants in water resources is unsatisfactory, largely because its importance has been recognized only recently. With literally thousands of pollutants entering water resources from municipal and industrial sewers and agricultural and urban land drainage, it is becoming increasingly necessary to be able to determine the course and amounts of these pollutants if control measures are to be devised and applied. Many pollutants in water have their origin in multiple sources. Man-made pollutants are intermixed with natural pollutants, including those resulting from natural chemical, biochemical, and biological reactions supported by man-made pollution.

Examples of concern are the origin of salt pollution (oil field brines, natural salt deposits, irrigation return flows); nutrients (agricultural fertilizers, sewage and organic industrial waste effluents, natural stream purification, chemical manufacturers); fecal streptococci (human, animal); mineralization (acid mine drainage, irrigation return flows, industrial wastes, water treatment); and many organic chemical wastes that must be traced to the specific manufacturing-plant outfall sewers.

In addition to controlling wastes, it is necessary to be able to prove the origin of specific pollutants in enforcement actions where the specific rules of evidence apply.

### Intramural Projects

(1) Improved methods for the quantitative estimation and interpretation of bacterial indicators of contamination.

The project is designed to provide more reliable and rapid methods for the quantitative enumeration, recognition, and interpretation of bacterial indicators of pollution. The ideal use of bacterial indicators of pollution requires that the technical procedure is quantitative; recognition is rapid and accurate; source of such microorganisms as feces, soils, insects, etc., can be determined; and, that limitations of the procedure are recognized.

Microorganisms with established values that indicate pollution are the "total coliform group," the "fecal coliform group," and the "streptococcal group." Further interpretations can be estimated with reasonable accuracy by the ratio of the total coliform to fecal coliform and fecal coliform to streptococcal group, and by the classification of the various species of streptococcal strains present.

The characteristic coliform bacterium from the gut of warm-blooded animals is established as one that, on a confirmation procedure, produces gas from lactose in a suitable medium in 24 hours or less at 44.5 ($\pm$ 0.5)° C. A membrane filter technique has been

developed for the fecal coliform group that appears to have sensitivity and specificity equal to the multiple tube procedure.

Investigations to establish the importance of storm water drainage from metropolitan areas are continuing. Preliminary results indicate that coliform, fecal coliform, and streptococcal groups are present; densitities are greatest in spring and summer; fecal coliform densities are relatively low; densities decrease with prolonged rainfall; and, the streptococcal strains are atypical with some resemblance to those from animals other than humans. There were, however, no Streptococcus bovis or S. equinus strains present.

Additional data have been obtained on ratios between the fecal coliform/fecal streptococcal groups from various animals that are useful in the interpretation of possible sources of pollution. The fecal coliform/fecal streptococcal group ratios for the following animals are: human, 4.3; duck, 0.61; sheep, 0.43; chicken, 0.38; cow, 0.18; turkey, 0.10; and pig, 0.04.

The streptococcal group is tentatively subdivided into: the fecal group that grows at 10° C and 45° C or that grows at 45° C only and the nonfecal group that grows at 10° C but fails to show growth at 45° C. Subsequent investigations may require a reevaluation of this definition, but current data support this procedure as the simplest method of classification. Growth at 10° C with no growth at 45° C is characteristic of streptococci found in cannery wastes.

Classification of the fecal streptococcal group by species is an involved and time-consuming process. The feces from humans essentially consist of four species that many investigators have described as the "enterococcal group." The feces of other warm-blooded animals contain S. bovis, S. equinus, and S. acidominimus. These data provide another means for differentiating human wastes from those of other warm-blooded animals.

(2) Origin of organic chemical pollutants in water.

This study utilizes the natural carbon-14 activity of recovered organic contaminants to distinguish between biological waste materials and chemical industrial wastes. Biological waste, such as sewage, contains a greater proportion of radioactive carbon due to its recent synthesis than does chemical industrial waste, which is derived from hydrocarbons containing very old carbon atoms. The procedure is now practical where relatively large quantities of organics, about 10 grams, can be recovered. It has been applied to the study of pollutant sources of three water supplies and has clearly distinguished petrochemical waste contaminants, equal proportions of industrial and biological wastes, and wastes primarily of biological materials. The method also assists the interpretation of the purely chemical analyses customarily performed on recovered organic materials.

(3) Field test of analytical techniques developed to demonstrate pollution.

This project consists of intensive field application of the newest analytical techniques to evaluate their utility in establishing damage to water quality. The study site is the Kanawha River in West Virginia, which carries a very heavy load of chemical industry wastes. The pollutants causing damaged uses of water downstream are demonstrated to be the same specific organics discharged into the river at a point upstream. Concurrent with newer sampling and analytical techniques, conventional sanitary-engineering field survey methods are applied to determine the extent to which they supplement or are supplanted by the new, specific organic analysis methods.

The results showed that deterioration of water quality evidenced by occurrence of taste and odor troubles, periodically occurred at points on the Ohio River below its confluence with the Kanawha River. These events were correlated with increases in the concentration in the Ohio of the same organics discharged into the Kanawha. When the problem was most severe, disagreeable odor was produced in the Ohio as far downstream as its confluence with the Mississippi.

Before the project is complete, an intensive survey will be made of other potential sources of the same contaminants along the Ohio and its other tributaries.

(4) Water quality attrition by municipal use.

This project was initiated to provide basic information on the amount of organic and inorganic load contributed by a municipality in one cycle of water use.

To provide these data several "grab" samples of tapwaters and secondary sewage effluents were collected from five Ohio municipalities, ranging from very small towns to highly industrialized cities. The samples were taken at peak loadings on different weekdays over a period of many months. The cities were selected so that the effluents would come from sewage treatment plants of different types and sizes and only from cities having a uniform water supply. Examples of average calculated increments are:

| | | |
|---|---|---|
| ABS | 6.9 | lbs/day/1000 people |
| Total nitrogen | 22.8 | " |
| Total phosphate | 22.3 | " |
| Soluble chemical oxygen demand | 79 | " |
| Calcium and magnesium | 30 | " |
| Chloride | 58 | " |

Contract Projects

(1) Application of the physical description of the hydrology of a large land mass to specific problems of water supply and pollution control.

The first work on this project conducted by the Rand Development Corporation has been the development of a mathematical representation of the general pattern of mineralization of surface waters. Analysis of data on dissolved solids and river discharge demonstrated that the relationship between the two remained functionally linear (log-log transformation) regardless of flow, topography, drainage basin size, and many other variables tested for many rivers in the country. The above relationships will be investigated for additional streams, and physical-chemical phenomena such as adsorption and ion exchange will be investigated as possible explanations of the relationships. The understanding of the relationship is expected to be useful in predicting effects of ground water vs. surface water on the mineral content of a stream, or in demonstrating pollution from other than natural sources by destruction of the linear relationship.

## IV. CONTROL OF WASTES AT THEIR SOURCE

### The Problem

Wastes can be broadly categorized as those that can be collected and conveyed to a central place for treatment, e.g., municipal sewage, urban storm drainage, and most industrial wastes, and those that might be best controlled at their source, e.g., acid mine drainage, natural and oil field brines, irrigation return flows, and other agricultural drainage. Algal blooms and other nuisance vegetative growths in lakes, streams, and impoundments and water quality deterioration that often results from water impoundments should also be controlled at the source or place of occurrence.

Industry has the responsibility for research in controlling its waste at the source through development of process changes, and use of new raw materials which eliminate or reduce pollution discharges. The Federal government more properly should concern its research in those situations listed above that are in the public domain.

Increased water use and growing competition between all water users have resulted in recent concern about these problems, and it is very apparent that source controls for acid mine drainage, agricultural land drainage, brines, impoundment water quality, etc. need to be developed soon.

### Intramural Projects

(1) Land drainage as a factor in stream pollution.

(a) Urban runoff study.

The study has acquired data for rainfall and runoff from the storm sewer serving a 27-acre, light-commercial urban drainage area. Several hundred analyses were made on samples collected during 42 storms over the year. These storms occurred during months of extreme dryness, extreme rainfall, and near normal precipitation. This situation allowed evaluation of the effect of antecedent rainfall on runoff water quality.

The chemical data show some effects of seasons on BOD, phosphates, and bacteria counts; all were much lower during the winter months. The total and volatile suspended solids, COD, and total nitrogen concentrations increased with the higher intensities of rain and consequent higher flows. Most of the parameters measured drop in concentration as a rain continues after the first 2 hours of runoff has occurred.

In the area studied, the annual pollution load in the receiving stream due to storm runoff could range from 20 to 400 percent (depending on the parameter chosen) of the pollutional load from the treated domestic sewage of the same area. Due to

the sporadic nature of rainfall, these values could increase as much as 10 times for any one day during which 1 inch of rain fell.

(b) Rural runoff study.

A cooperative study has begun with the U. S. Department of Agriculture's Soil and Water Conservation Research Station at Coshocton, Ohio. Five of that station's watersheds have been selected for study because of their size, accessibility, and land management (improved practice or prevailing practice). These practices differ mainly with respect to tillage and fertilizer, manure, liming, and pesticide applications.

In the few runoff samples obtained so far, large variations in water quality were observed among watersheds and in relation to the amount of rainfall.

(2) Chemical control of algae.

Studies were begun to develop new methods for determining algicidal or algistatic properties of chemical compounds reputed to have these qualities. Initial experiments involved combining 2 mg/l of the test compound with several species of algae to determine whether or not it warranted detailed experimentation. Six compounds were tested with species of green algae, blue-green algae, and diatoms.

All the compounds were algicidal to the diatoms and blue-green algae. Since the green algae were more resistant to all of the compounds, work was done to find a compound that would be algicidal at a low concentration to a large number of species of green algae. One of the six (di, N, N dimethylocoamine salt of 3, 6 endoxohexahydrophthalic acid) was found to be algicidal to seven of the eight species tested at a concentration of 2 mg/l after 15 days incubation. Different methods for evaluating the amount of cell growth were explored. Optical densities of cultures measured with a Beckman electrospectrometer compared with dry weight determinations of aliquots of test cultures proved the latter is a more reliable method of measuring the amount of cell growth. A Coleman Photo-Nephelometer that measures the concentration of extremely dilute suspensions was recently employed and, when calibrated against mg/l dry weight of culture sample, proved to be a relatively accurate and simplified method of measuring the amount of suspended cells in test flasks.

(3) Biological control of algae.

(a) Research to discover algal viruses and investigations of their possible use in algal control.

Environments with dense algal populations have been under study for the past 2 years to determine the possible existence of

algal viruses. Recently, water samples collected from a waste stabilization pond were found to lyse members of the blue-green algal group. A virus has since been isolated from the sample and found to lyse 11 filamentous algal strains. Included in these forms were members of three genera, Lyngbya, Plectonema, and Phormidium. Recent studies indicate they may belong to one genus.

Electron micrographs of a partially purified preparation show the viral particle has an icosahedral structure about 66 mμ in diameter. Thus, the virus is morphologically similar to the adenovirus and wound-tumor virus, a large number of other animal and plant viruses, and at least one insect virus. The virus can be assayed by the plaque method and, on propagation of the virus, titers of about $10^8$ particles per milliliter of medium are not uncommon.

(b) Research to discover, evaluate and identify natural algicides; determination of the chemical nature and potency of an algicidal agent recently isolated from a natural product.

A highly promising antialgal agent isolated from a soil actinomycete has been partially purified through treatment with activated carbon and organic solvents. The substance, when tested against a large number of bacteria, actinomycetes, fungi, and algae was found to be specific for the blue-green algae. At a concentration of 3 mg/l, a partially purified preparation of the algicide completely inhibited the growth of Anacystis nidulans. Full-grown cultures of Anacystis nidulans showed complete lyses of the cells at 10 mg/l of the algicide.

(c) Determination of antagonistic effects of actinomycetes on algae found in waste stabilization ponds.

The basic relationship between antibiotic formation in nature and the nutritional constituents of the environment suggested that the antagonistic effects on algae observed in waste stabilization ponds may be partly due to the action of antibiotic substances. Studied was a quarter-acre experimental pond that was operated at a 3-foot depth and received domestic sewage at the rate of 120 pounds of BOD per acre per day. Its actinomycete population, determined by plate counts on egg-albumin agar, was found to be negligible in the pond water; whereas, in bottom and bank muds, actinomycete counts ranged from 600,000 to 30,000,000 organisms per gram dry soil, depending on the origin of the sample. It was found that 51 percent of the organisms isolated were active against the algal test organisms. Four antibiotically-active pond isolates, selected at random, were investigated in soil perfusion units containing water and mud deposits from the pond. After an incubation period of 14 to 21 days, the extracellular products of these cultures exhibited

antibiotic activity against either Anacystis nidulans or Scenedesmus basilensis or both when assayed by the paper disc method.

## V. TREATMENT OF WASTES

### The Problem

Present treatment methods for both municipal and industrial wastes were devised for problems that existed 40 years ago and are based fundamentally on mechanical and biological principles. These processes, at best, provide only partial treatment; they are proving increasingly inefficient for today's waste concentrations and are entirely inadequate for many of the new synthetic chemical wastes. Moreover, satisfactory treatment methods were never devised for many of the older types of wastes that are now produced in quantitites proportionate to the very great increase in manufacturing, i.e., pulp and paper, metal finishing, food processing, and textile wastes.

Water treatment is considered to be an extension of waste treatment since its function also is the removal of contaminants. Water treatment processes, developed long ago, are not designed to remove the contaminants in solution. Hence, raw waters contain increasing amounts of soluble pollutants such as synthetic detergents, pesticides, and organic chemicals, and these substances are reaching the consumer-public in ever larger amounts through its drinking water. Although present levels are relatively low and no acute or chronic effects on humans have been demonstrated, industrial production forecasts for these substances show a real need to improve the effectiveness of water and waste treatment processes.

### Intramural Projects

(1) Behavior of organic chemicals in biological treatment.

(a) Study of extended-aeration sewage treatment plants.

The effluent quality was much better during controlled wasting of suspended solids from the test units. The optimum solids concentration and amount of wasting varied with operation temperature, flow characteristics, loading, and other factors. High-solids concentrations at summer operation temperatures produced a stabilized sludge that was low in volatile content and had poor flow characteristics. During this time high-solids effluents were common. Operation temperatures below 10° C produced more sludge at a lower density and very poor drainability. Additional sludge stabilization was indicated but effluent quality was good if the suspended solids were held below about 5,000 mg/l.

(b) Anaerobic treatment of organic chemicals.

Addition of acrolein sufficient to provide a concentration of 50 mg/l in a digester prevented gasification after two or three doses. Lower dosage permitted reduced gasification of a reference feed, but the chemical appeared to volatilize rather

than digest. Allyl chloride produced similar behavior with a maximum tolerance limit below 30 mg/l. A white polymerization or degradation product accumulated in the gas holder during sustained allyl chloride dosage. Some adaptation of organisms to the chemicals was noted at dosages below the cited tolerance limits.

(c) Effect of chlorides on biological systems.

Gas production during anaerobic digestion of a reference feed was about 80 percent of normal at 20,000 mg/l of chloride when the digester chloride level was increased at about 500 mg/l per week.

The activated-sludge process was similar to anaerobic digestion in that performance was not significantly affected when the chloride level was below 7,500 mg/l. Above this level of chloride concentration, the performance varied with the rate of change in chlorides and stabilized within 1 to 3 days after reaching a given chloride level. Studies are continuing.

(2) Nutrient removal studies.

Treatment of digester supernatants in a heated packed column by means of lime, caustic, and aeration shows that it is possible to reduce the ammonia concentration from more than 400 to about 40 mg/l and the phosphorus concentration from above 40 to less than 4.0 mg/l. The phosphorus was precipitated with lime as calcium phosphate and settled; the supernatant was treated by aeration at pH 10 or above at a temperature exceeding 70° C. Lime used for pH adjustment resulted in a large pH drop before or during treatment in the heated column. The pH drop was minimized when lime and caustic were used or when prior acid treatment was used to destroy bicarbonate buffer capacity.

A modification of the activated sludge process to provide intermittent aeration was not considered advisable for control of effluent nitrogen either in the extended aeration process or as a substitute for the semiaerobic process. The conditions for good nitrification and denitrification at the same time were difficult to maintain.

(3) Effects of heavy metals on sewage treatment processes.

Previous studies have been concerned with the effects of copper, chromium, nickel, and zinc when introduced singly into the sewage feed of activated-sludge pilot plants.

Investigation of the effects of these metals in combination has not been completed. A combination of four metals, totaling 8.9 mg/l, had no great effect on the overall efficiency of a pilot-scale

activated-sludge plant. No synergistic action, in the range studied, was noted. No difficulty was encountered with anaerobic digestion of the sludges produced by the plant. The distribution of the metals through the process was traced, and it was found that approximately 90 percent of the zinc, 54 percent of the copper, 31 percent of the nickel, and 37 percent of the chromium was removed from the influent sewage. The metals, in combination, behave independently in their distribution through the process.

Nitrification in the unit that was fed metals was almost completely inhibited. This was a general symptom of heavy metal toxicity and not due to chromium specifically.

The metals passing through the activated sludge process and discharged with the final effluent, with the exception of zinc, are predominantly in a soluble form.

(4) Studies of water purification procedures: Review and development of water plant design.

Studies, employing the pilot plant facilities and turbid water from the Little Miami River, to determine the influence of various major changes in plant design and operation on removal of gross turbidity, coliforms, powdered activated carbon, poliovirus, and odorous organics have continued.

A double-layered filter bed composed of 18 inches of coarse coal on top of 6 inches of sand was able to remove as much or more turbidity, coliforms, poliovirus, or powdered activated carbon as a bed of coal or sand alone. Such a bed also permitted the extension of filter runs.

Additional evidence was obtained to demonstrate that, with proper coagulation ahead of the filter, a filtration rate of 6 gpm/per square foot was as effective in removing all the particulates investigated as a rate of 4 or 2 gpm per square foot.

Adequate floc strength was more important than settling velocity to achieve clarity when coarse media and high filtration rates were used. This strength frequently was obtained only by adding 5 to 20 mg/l of activated silica as a coagulant aid or 0.05 to 0.2 mg/l of a synthetic polyelectrolyte as a filter aid.

When the water was relatively clear (40 J.u.), the flocculation and sedimentation steps of conventional treatment design could be omitted if coarse media were placed on top of some sand. For this particular river water, the use of flocculation and limited sedimentation permitted longer filter runs and better water during winter and flood conditions.

Beds of fresh, clean, granular activated carbon removed poliovirus in clear water more readily than beds of sand. Carbon that was exhausted by a high dissolved organic loading, however, did not remove much more virus than a similar-sized sand or coal bed.

A pressurized bed of 10 x 30 mesh, granular activated carbon 10 feet deep removed odorous material from tap water for 9 months when the feed rate was as high as 1 gpm per cubic foot of carbon. One mg/l of ozone ahead of the bed seemed to double the life of the carbon when there was a heavy odor load with a 5- to 20- threshold odor number. Similar doses of hydrogen peroxide did not extend the carbon life. With high flow rates and coarse carbon, there is always some leakage of dissolved organics; it is apparent that finer carbon and longer contact time will allow a greater use of the adsorptive capacity.

(5) Role of fungi in environmental sanitation.

(a) Laboratory guide.

The "Laboratory Guide for the Culture and Identification of Fungi in Polluted Water, Sewage and Sewage Treatment Systems" is available for distribution. More than 300 species of about 125 genera of fungi are "keyed out," and known pollution habitats are given for each. Camera lucida line drawings, mostly from fresh preparations, illustrate 116 genera. In addition to aiding identification of common fungi, this guide gives mycological literature a new source of original illustrations of many genera whose identifications previously were based on illustrations up to 30 years old.

(b) Development of a yeast quantitation procedure.

Decimal dilutions of the sample (1 ml) are inoculated into flasks that are incubated at room temperature on a shaker for 64 hours or as "still cultures" on the laboratory bench. The resulting growth for a single shaken flask can be stated in terms of the "indicated number" (reciprocal of the highest dilution showing growth) and for the multi-flasked (up to five) still culture as "most probable number" (MPN). In each technique, somewhat different populations may appear. Two major types of yeast populations are observed by these methods: those with red pigment, Rhodotorula, and those without pigment. By applying additional laboratory procedures, identification of the various genera and species can be made.

(c) Waste stabilization ponds.

From samples collected at the Lebanon waste stabilization ponds, 207 species of fungi were isolated and identified; 35 of these species were found in all samples examined. This indicates that relatively large numbers of fungal species are

able to develop in the presence of considerable organic pollution. The fungal population of the original soil species was increased by addition of raw sewage as well as by precipitation from the "air spora." The fungal populations in all samples were similar; they differed mainly in species distribution rather than composition.

(d) Fungi from the Cache Valley in Idaho and Utah.

In the 67 categories of fungi identified, there was an interesting relationship between populations observed and enrichment levels of the habitats. Differences were noted in the populations of the upper, clean reaches of the Bear River and the middle, more polluted, portion.

Differences were found between populations in the Logan Canyon samples and those in the Bear River series. These are apparently related to types of land use; the Logan Canyon area, which is undisturbed, has been used for grazing and recreation and the Bear River area is intensively cultivated.

(e) Molds and yeasts in sludges.

Studies were initiated to determine the fungal populations of primary settled sludge, settled activated sludge, and the same sludges after digestion. Preliminary results indicate that larger numbers of yeasts and molds are present in the settled sludges than in the digested sludges.

(6) Adaptation of microorganisms to degrade wastes and the relation of molecular structure to ease of degradation.

Results on the ability of a phenol-adapted culture to degrade structurally related compounds have been compared with recent results from cultures adapted to one of 12 other related compounds. Based on results presently available, the phenol-adapted culture has much greater ability to degrade related compounds than any other culture tested. This culture degraded 78 of the challenge compounds and was the only one that degraded 24 of them; 21 compounds were not degraded by any of the test bacteria.

Of all classes of compounds tested in considerable numbers, the benzoic acids and benzaldehydes were the most readily degraded. The nitrobenzoic acids were definitely the most resistant.

There was strong indication that molecular structure is related to degradability of certain classes of compounds. Benzaldehyde and benzoic acid were both more resistant to degradation than their respective p-hydroxy derivatives; 2,5-dihydroxybenzoic acid was not degraded by either the catechol or m-nitrophenol cultures while 3,4-dihydroxybenzoic acid was readily degraded by both.

A particularly outstanding example of the effect of position of substitution was observed in tests with o-, m-, and p-toluic acid. These are three isomers of the same methylbenzoic acid and differ only in the location of the methyl group on the benzene ring. Each isomer was tested with cultures specifically adapted to degrade phenol, catechol, resorcinol, phloroglucinol, o- and p-cresol and m-nitrophenol. The m-isomer was rapidly degraded by cultures adapted to phenol and p-cresol but it was not broken down by any of the others. The o- and p-isomers were not degraded by any of the cultures.

Catechol and quinol, which are respectively o- and p-hydroxyphenols, were efficiently degraded by a culture adapted to a markedly different phenol, p-nitrophenol. This culture did not degrade resorcinol, the m-isomer. The ability to degrade catechol and quinol apparently is not a characterictic common to all nitrophenol-adapted bacteria because the m-nitrophenol-adapted culture failed to degrade catechol, a compound that the p-nitrophenol culture degraded very rapidly, and only had limited activity with quinol.

The age of a culture and the pH and temperature at which it was grown, as well as the pH and temperature in respirometric tests, were shown to make the difference between success and failure in degrading some of these compounds. Investigation of these factors is currently being expanded.

(7) The identification of genera and species of algae in sewage stabilization ponds.

Samples obtained at weekly intervals from a number of widely scattered sewage stabilization ponds throughout the country have been analyzed for the kinds of algae present. It is becoming evident that the genera that remain in large numbers for any long period of time are a few pigmented flagellates and non-filamentous green algae. In addition to these, each pond may have a flora of other less abundant algae that readily distinguishes it from algae of other ponds even in the same locality.

## VI. ADVANCED WASTE TREATMENT AND WATER REUSE

### The Problem

With a relatively fixed quantity of water available for dilution, a higher degree of treatment or removal is necessary to maintain the same quality of receiving water as the amount of waste increases. Some present treatment methods have practical limitations as to levels to which pollution can be reduced. Present treatment methods do not satisfactorily remove some new pollutants. In water-short areas where deliberate reuse of water is to be practiced, new methods must be developed and applied to maintain and add to our water resources. Because each water use adds refractory wastes that are not removed by biological treatment, new treatment processes, probably based on entirely new concepts and principles, must be developed to renovate waste water to fresh water.

The pollutants of concern damage water quality and create aesthetic taste and odor problems in drinking water, off-tastes in fishflesh, color, and troublesome foaming. Economic damage results from corrosion, scaling, ion exchange deterioration, and excess costs to remove hardness and undesirable taste or odor. Physiological effects include fish kills, damage to aquatic life, and possible harm to human health.

Two important objectives must be achieved if the total concept of advanced waste treatment is to be feasible. First, it will be essential to concentrate the impurities in municipal waste water into small volumes and provide for permanent disposal of this concentrate. Second, it will be necessary to produce a quality of purified water suitable for direct reuse. Concentrated wastes removed by advanced waste treatment processes cannot, of course, be discarded into usable surface or ground waters or the same contaminants would have to be removed again and again. The development of methods for permanently disposing of concentrated impurities is, therefore, an important part of the concept. Efficient removal of contaminants will result in the production of water of excellent quality. Provision should be made for deliberate direct reuse of this water by the community, by industry, by agriculture, or in recreational facilities since, in most cases, it would be wasteful to discharge an excellent, high-quality water into a stream that is less pure, perhaps much less pure, than the treated discharge. The development of a successful advanced waste treatment technology, therefore, could lead to the alleviation, in a single step, of both water pollution and water supply problems.

From a technical standpoint, advanced waste treatment may be looked upon as a two-step process: separation of concentrated contaminants from the purified water product and disposal of the concentrated contaminants in a way that renders them forever innocuous. Research is necessary, therefore, on both separation and permanent disposal techniques. These two elements of advanced waste treatment are related economically since, in general, the more a waste is concentrated, the higher the concentration cost but the lower the disposal cost.

It is conceivable that almost any physical-chemical principle of separation is applicable to advanced waste treatment. Principles under active con-

sideration include adsorption, chemical oxidation, coagulation electrodialysis, electrochemical conversion, eutectic formation, evaporation, extraction, reverse osmosis, foaming, freezing, and ion exchange. None of these unit operations has been applied to a significant degree in municipal waste treatment. Early results in this area have already led to several pilot applications of these techniques and even to proposed plant designs.

Intramural Projects

(1) Foaming.

A continuous foaming operation has been shown to remove ABS to acceptable levels and significantly remove other organics. Plans have been made to investigate foaming of waste waters on a pilot-plant scale. Part of this study will involve combining the foaming of secondary effluent with recycle of the organic-rich collapsed foam to the secondary treatment influent. This method of operation has been suggested as a way of increasing the residence time of refractory organics in the secondary process.

The addition of certain polyelectrolytes to waste water before foaming has resulted in very good removal of organic materials from both primary and secondary effluents. Decreases in COD of 70 percent have been obtained. Further laboratory studies of this aspect of foaming are underway to find the best additive and the optimum amount necessary.

Since foaming is a cheap organic removal method, it should be attractive to a number of industries as a method of treating their waste water before discharge to a sewer.

Thus far, organic removal by foaming has been measured only in terms of COD and ABS. A better idea of what is being removed is desirable. A detailed chemical analysis of organics removed by foaming or left behind after foaming would be impractical. A characterization of waste water before and after foaming in terms of properties such as color, odor, and toxicity to fish is, however, practical, and such a study has been undertaken.

A field study of foaming has been undertaken at Cleveland's Easterly Water Pollution Control Plant to determine what improvement is possible in the effluent from a secondary treatment aeration basin when the generated foam is removed rather than collapsed. Measurements have been made of the rate of foam production over an aeration basin, and devices for continuously removing the foam are under study.

(2) Oxidation.

A detailed study has been made of how the COD of secondary effluents can be decreased by adding catalysts and hydrogen peroxide

as an oxidizing agent. Reductions in COD as high as 85 percent were observed. The process, however, is not economically feasible at present to treat waste waters. Other oxidation systems are being investigated.

Contract Projects

Since in some cases more than one contract has been negotiated for study of different phases of a process, the specific contractors involved on these projects are not identified.

(1) Evaporation.

Techniques for evaporating waters have been greatly improved in the last few years. The evaporation process is of interest in waste-water renovation because both organics and inorganics are removed. Although volatile materials may pose problems, these problems can almost certainly be solved.

A desk top study of scaling has indicated that distillation temperatures as high as 350° F should be possible with many municipal effluents. The use of such high distillation temperatures should result in lower overall costs for this water than the cost for converted sea water. Continuous boiling experiments with secondary effluent from Ann Arbor, Michigan, indicate no serious inorganic scaling or organic fouling problems when temperatures up to 250° F were used. pH adjustment to below 6 was necessary to prevent carbonate scale.

A pilot plant investigation of evaporation with a multistage flash evaporator is now underway. During the course of this investigation, a study will be made of the effect of scaling on heat-transfer rate and the effect of foaming and volatiles on product quality for a wide range of temperatures. From the results, a realistic assessment of the feasibility of waste-water evaporation should be possible.

(2) Electrodialysis.

The ability to lower the inorganic ion content of waste water to an acceptable level by electrodialysis has been established. This process has not proved effective in the removal of organic ions. It is quite likely, however, that organic materials will be removed from the waste water prior to treatment by electrodialysis.

In the early experiments, no attempt was made to obtain a low ratio of waste-water volume to treated-water volume. Since a small waste volume is desirable in waste-water treatment, a study has been undertaken to determine the minimum possible volume of waste stream. Preliminary results indicate a waste stream of 2 or 3 percent of the feed volume might be possible.

To better assess the technical feasibility of electrodialysis and to get a preliminary estimate of its economic feasibility, tentative plans for a pilot plant unit have been prepared.

(3) Ion exchange.

Ion exchange, like electrodialysis, could be an effective method for removing inorganic ions from waste waters. The cost of chemicals to regenerate the ion-exchange resins has been partly responsible for making the process prohibitively expensive. A system that recovers the regenerating chemicals has been proposed and is being investigated. The system involves nitric acid as a cation resin regenerant and ammonium hydroxide as the anion resin regenerant. Evaporation of the cation resin regenerant brine and calcination of the remaining residue allow nitric acid to be recovered and give a mixture of metallic hydroxides. The hydroxides can be used in recovering ammonium hydroxide from the anion resin regenerant brine. The technical feasibility of the system is being studied.

(4) Electrochemical processes.

The electrolytic treatment of waste water has been investigated. Significant reduction of a number of contaminants including organic materials was obtained by use of direct current and several kinds of electrodes. The process, however, does not appear economically attractive.

(5) Adsorption.

Granular activated carbon has been shown to be effective in removing nearly all the organic materials from waste water. Thermal regeneration of the activated carbon that has been loaded with organic contaminants is technically feasible. Although only a very preliminary estimate of the cost of organic removal by carbon adsorption has been made, the economics of the process appear very promising. Detailed paper studies of a number of adsorber configurations and reactivating furnaces will be made to obtain a better cost estimate and to develop a pilot plant unit.

Theoretically, powdered carbon rather than granular carbon should allow a higher contaminant loading. Handling powders is more difficult, however, than handling granular material. An experimental investigation will be made to determine the feasibility of adsorption with powdered carbon.

Although adsorption by granular carbon removes most of the organics from waste water, there remains enough organic material to exert a COD from 10 to 15 mg/l. A study is being made of the composition of these residual contaminants so that a method for their removal might be found. Screening of a large number of activated carbons is also being undertaken to determine if carbons of different origin remove different amounts of contaminants.

Although activated carbons are too expensive to use but once, a number of coal products and coal wastes are being studied to learn if any of these materials might be used on a onetime basis. If the adsorbent had a significant fuel value, a onetime use might be feasible. The adsorption results with coal and coal products have, thus far, been much less encouraging than results with activated carbon.

(6) Freezing.

A contract is now in effect for further investigation of the technical and economical feasibility of renovating waste waters by the freezing operation. The contractor is using a continuous freezer-washer-melter system with a feed capacity of about 15 gallons per day. Preliminary runs have indicated that the ice produced from waste water is quite different in physical appearance and behavior from ice produced from a sea-water solution. The ice produced from sea water is crystalline in nature and does not seem to agglomerate easily; that produced from waste water leaves the freezer in agglomerates up to one-half inch in dimension. These larger particles of ice seemingly cut down on washing efficiency. In a series of three passes of a typical waste water through the equipment, the contractor found that the concentration of contaminants increased from pass to pass, as would be expected. From pass 1 to pass 3, the product ABS concentration increased by a factor of 1.7, the COD by a factor of 2.4 and the salts by a factor of 5.2. It appears that washing was more successful in keeping the COD and ABS levels down than in lowering the salt content. This indicates that ABS and COD can be washed from the ice at least as effectively as is the salt. Therefore, if a pure, small particle ice can be formed, the organic contaminants should not present a washing problem.

In an attempt to decrease the concentration of contaminants in the product, the contractor has installed an ice crusher between the freezer and the washing column. The objective is to produce smaller particles of ice that should wash more efficiently. This innovation has increased the efficiency of the washing operation, but the product concentrations will contain about 20 mg/l of COD and 1-1/2 mg/l of alkyl benzene sulfonate. The cost of the crushing operation has not been estimated.

In another contract on freezing, the contractor, investigating eutectic freezing or eutectic gas hydration as a preconcentration step before ultimate disposal, has determined that both freezing and gas hydrate eutectics do form and that the ice and contaminant crystals form separately and can be separated. The feasibility of salting out organics and other inorganic materials as the concentration of sodium chloride increases is now being investigated. The effect on inorganic contaminants is being investigated in a small scale unit, using waste water as the feed. The feasibility of salting out organic contaminants is being investigated using alkyl benzene sulfonate and sodium chloride solutions in test tubes.

(7) Extraction.

A 1-year contract has been completed on a study of the feasibility of waste water renovation by extraction with amine solvents. In this process, the water complexes with the solvent and is removed, leaving the impurities behind. To date, the contractor has determined that a continuous extraction column (laboratory scale), using di-isopropyl amine as the solvent, will yield a product containing about 30 mg/l COD, 0.8 mg/l ABS, and 20 mg/l chloride. The phosphate evidently was completely removed, but the nitrates were not affected by the process. During continuous operation, the change in the physical nature of the solvent was such that the flow rates had to be reduced considerably to prevent flooding. Another cause of this was gradual fouling of the packing material. It was determined that essentially all of the detergent and about 20 percent of the organic material in the feed remained with the solvent phase. The contractor is now studying regeneration of the solvent, coagulation of the feed to remove the fouling contaminants, the effect of the process on various inorganic ions, and the advantages to be gained from using other solvents for both organic and inorganic removal.

(8) Reverse osmosis.

Reverse osmosis entails overcoming the osmotic pressure of a solution and thus forcing relatively pure water through the membrane. A contract was negotiated late in fiscal year 1963 for a 7-month investigation of this process. In preliminary work, it had been shown that existing membranes will exclude both organics and inorganics from synthetic solutions. It is expected that a decision will be made on the technical and economical feasibility of this process after the short investigation.

(9) Ultimate disposal.

It has been stated that the contaminants separated from the waste water must be disposed of so as to render them forever innocuous. This is termed "ultimate disposal". Previous studies in this field have investigated injection into deep wells, disposal into underground cavities, wet oxidation, incineration, and ground spreading. Detailed plots of costs versus capability have been prepared for these processes. It appears, in general, that the expense of ultimate disposal will be minor as compared to the purification process costs.

## VII. DISPOSAL OF WASTES INTO THE WATER ENVIRONMENT

### The Problem

While wastes have been discharged into surface water, e.g., rivers, streams, lakes, ground water, and coastal water for many years, little is known about the fate of pollutants and the capacity of these waters to receive wastes. This is becoming an increasing problem for the 23 states located on coastal waters, the 8 states located along the Great Lakes, and the larger cities on the Nation's streams. To make the most effective use of augmented streamflows from impoundments, information is needed about quality changes that occur in stored water and minimum quality conditions obtainable by control. Research pertaining to surface waters is an integral part of a project of broader scope and is included in the next section.

Because several million persons depend on septic tanks and soil adsorption systems for waste disposal, the basic principles of waste disposal in waters and soils must be defined and applied.

Investigations have shown that use of the soil mantle to assimilate wastes such as virus and ABS can be improved considerably through proper design, construction, and operation. In areas where surface water disposal is not available, satisfactory environmental conditions may be preserved through proper disposal to the ground.

### Intramural Projects

(1) Factors influencing the design and operation of soil systems for waste treatment and disposal.

(a) Experiments with pilot-scale disturbed-soil lysimeters for treating septic settled sewage support the following findings:

1. The seeding that is necessary in soil systems to handle biologically resistant organics can be achieved from either the sewage, soil, or air. It usually takes 2 or 3 weeks to achieve a maturation that will permit rapid oxidation.

2. Additives, such as activated carbon, vermiculite, or organic soil, will help prevent initial passage of contaminants by virtue of their high adsorptive capacity.

3. The transfer of one-half inch of soil from the top of a mature bed to a new bed will also prevent high initial penetration of contaminants. It is particularly effective in starting early nitrification.

4. A low hydraulic rate (1 gpd/per square foot) during the first month of operation is a third technique for preventing early passage of ABS, ammonium, and COD. With sandy soils, higher rates (up to 5 gpd/per square foot) can be used thereafter.

5. As expected, lower temperatures reduce the metabolic rate, but a 55° F condition can be handled by reducing the initial rate or by using additives. The maturing period still takes 6 instead of 3 weeks. A 40° F condition prevents effective maturation.

6. Failure is easily caused by overfeeding, but recovery is readily accomplished by mixing the top 2 or 3 inches of soil. Rest or rate reduction was not very effective in bringing about recovery.

7. Feeding the soil system intermittently six times per day will speed up the oxidation and nitrification, although after maturation a once-a-day loading will do as well.

(b) Studies with rigid porous membranes of known physical characteristics employed to stimulate soils reveal the following:

1. Soil clogging or the rate of use of soil area under subsurface application is directly proportional to organic loading. For example, units dosed with septic sewage diluted by 50 percent have developed only half as much wetted area as undiluted sewage.

2. Available soil spreading area can be used more efficiently by increasing the rate of hydraulic loading during an application period and decreasing the frequency of application.

3. Rate of degradation of organics in an infiltrated waste is inversely related to the rate of soil clogging.

4. On the order of 40 to 50 percent of input nitrogen is unaccounted for in soil flowthrough systems.

Contract Projects

(1) Domestic waste treatment potential of soil.

A research contract study with the New York State Department of Health to investigate the fate of pollutional materials in the unsaturated soil zone surrounding cess pool systems in the Long Island area has just been started.

## VIII. WATER RESOURCE CONSERVATION AND QUALITY MANAGEMENT

### The Problem

The severe drouth in the Southwest during the 1950's and other less extreme ones in other parts of the country in recent years have demonstrated the close relationship of water quantity and quality. Obviously, if the amount of pollution remains constant as the quantity of water decreases, water quality must deteriorate. The development and utilization of techniques of operations research and systems analysis will help establish, by taking into account the quality requirements for the different water uses, the most effective allocation and use of our water resources.

Emphasis was given this problem when Congress amended the Federal Water Pollution Control Act in 1961. Included was a directive to conduct the research necessary and develop methods..."for evaluating the effects on water quality and water use of augmented streamflows to control water pollution not susceptible to other means of abatement."

In support of research by other agencies relating to reservoir evaporation control, information is needed about prevention of bacterial decomposition of the chemical compounds that form in monolayers to increase the effectiveness of the monolayers customarily employed.

### Intramural Projects

(1) Effect of streamflow regulation on water quality.

The purpose of the project is to determine the effect on water quality of impounding water before its release for streamflow regulation and to determine the effect on downstream water of these releases.

(a) Laboratory experimentation.

A 500-gallon, 10-foot deep water tank was constructed to simulate an impoundment. By insulating the tank, cooling the bottom waters, and heating the top diurnally, a stratified condition similar to that in a deep reservoir or lake was simulated. The top half of the water was homothermal, followed by a sharp thermocline with the maximum gradient 7° F per foot. Finally, the bottom foot of water was uniformly cold, 52° F. Tracer studies, with $NO_2$-N as the tracer, showed the top water mixed about once every hour; none of the tracer introduced at the top reached the cool bottom water after 6 days. Surface mixing and wind action was simulated by a laboratory mixer and a stream of compressed air, and radiation was provided by diurnally operated "Gro-Lux" bulbs.

This tank behaved like a stratified impoundment chemically as well as thermally. During the 90-day test, the dissolved oxygen concentration rose steadily in the top waters but remained zero in the bottom waters. The COD concentration fell in the top waters but remained high near the bottom. Soluble iron and manganese concentrations were quite high just above the bottom muds but were low in the surface water. The behavior of these as well as other chemical constituents indicated that this type of laboratory apparatus will be a useful tool to study, under controlled laboratory conditions, rates of change of water quality parameters during storage.

A second laboratory project to study the nitrogen cycle in river water in an effort to make a complete nitrogen balance, including any loss of nitrogen gas, was started just at the end of the fiscal year.

(b) Field experimentation.

Considered but not pursued at this time was the continuation of a project, begun in 1958 by the Technical Advisory and Investigations Section, on the possibility of mixing large bodies of water with relatively little power input. Attention was turned, instead, to an investigation of oxygen relationships in impoundments. Some recent studies have indicated that in slowly moving waters, photosynthetic oxygenation is as significant as atmospheric aeration in the over-all re-aeration picture. Past measurements that indicate this are suspect, however. A special dissolved-oxygen probe apparatus was designed and purchased for further investigations.

Basically this is a three probe unit, one probe enclosed in a clear lucite box, the second in a dark lucite box, and the third external to both boxes. The open boxes can be lowered to any depth and closed to contain water from that depth. The observed rate of change of dissolved oxygen concentration measured with time would then be correlated to environmental conditions. The great difficulty experienced with the unit has forced redesign by the manufacturers who claim they will eventually meet specifications.

(2) Evaporation reduction.

(a) Instrumentation.

An instrument combining a dual system reflectophotometer for measuring evaporation efficiency and an electrophotometer for measuring the film pressure of monolayers on water was standardized and studied for its chemical and (or) physical characteristics. The soundness of the physico-chemical principle embodied in the reflectophotometer is demonstrated by the fact

that the rates of water evaporation obtained by both the half and the dual system follow closely the vapor pressure within a temperature range of 6° to 40° C. At temperatures about 40°, the temperature-hydration time curve levels off due to the formation of bluish $CoCl_4$. In the presence of a hexadecanol monolayer, the temperature-$CoCl_2$ hydration time curve bulges slightly in the 20° to 30° range, which indicates a maximum evaporation suppression efficiency (ESE) at about 25°. The results show that the ESE of a monolayer film like hexadecanol can be ascertained in about 10 to 20 minutes at temperatures between 18° and 30°, that an ESE of as little as 8% can be detected, and that the maximum ESE of hexadecanol film is about 45%.

Results with the electrophotometer, used to measure the film pressure of monolayers, yield a film-pressure-time curve, the profile of which closely resembles that from direct readings from the hydrophil balance. By the use of a calibration curve obtained with known weights, the equilibrium pressure of a known film can be used from a recorded curve.

Data for hexadecanol show that as the film pressure of the monolayer drops below 30 dynes per centimeter, the ESE becomes insignificant. High values of efficiency are obtainable at film pressure above 35 dynes per centimeter.

(b) Means to prolong film life of monolayers.

Silver stearate was prepared in the laboratory and used as an additive to the hexadecanol or octadecanol to retard degradation of the monolayer by Pseudomonas, Flavobacterium, or Zooglea ramigera. The last organism appears to be more damaging to monolayers, due to its preference to grow on water surface; but its growth rate is so relatively slow that it tends to disappear when the others are present. In daily application of a mixture containing 99.8% alcohol and .2% Ag-stearate, good ESE and film pressure were maintained during a 2-week period. The treated water surface remained clean over 95% of the area; but the bacterial population in the water showed very little change.

The hexadecanol-$I_2$ complex was studied for its physicochemical properties by X-ray defraction and spectrophotometrically, to determine the effect of the $I_2$ on the structural arrangement of hexadecanol. Its ability to retard bacterial degradation of the alcohol in monolayers is being studied.

The use of a 0.2% Ag-stearate as an additive in the fatty alcohol mixture in daily application for evaporation control has been recommended to the Bureau of Reclamation for use in field tests this summer.